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QUANTITATIVE STUDIES OF THE FACTORS GOVERNING THE RATE OF REGENERATION IN TUBULARIA

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The purpose of these investigations is to determine the reciprocal effect of two regenerating regions in *Tubularia*. In order properly to design the experiment it is necessary first to work out the rates of regeneration of different parts of the stem and to determine the effect of size of stem on rate of regeneration. Following this two regions of known rates are allowed to compete with one another and the effect on rate of regeneration is measured. Since it is found that the region exhibiting the lower rate is most affected, further experiments are designed to measure this effect. This inhibitory effect exercised by the higher rate over the lower rate has been termed physiological dominance by Child (1929) and the term "dominance" is used in this paper to describe inhibition.

When the stem of *Tubularia* is sectioned the hydranth differentiates *in situ* from the tissue of the cut end and after 30–40 hours at 18° C. the length of the primordium can be measured by means of an ocular micrometer. At this time a constriction appears at the base of the developing hydranth separating it from the rest of the stem. The perisarc being rigid, the diameter of the hydranth can be measured and the volume calculated. The time for regeneration is measured at two stages of development. t_1 is the time in hours from the time at which the stem is cut to the time at which the constriction appears between the primordium and the rest of the stem. t_2 is the time for complete regeneration when the hydranth is pushed out of the opening of the perisarc. In some experiments where short pieces of stem are used it is necessary to use t_1 as a measure of time since the hydranth, although completely differentiated, does not emerge from the end. In most cases, however, t_2 is used for the calculation of rate of regeneration.

Rate of regeneration can then be defined as the volume of the hydranth in cubic micra divided by the time in hours. $R = \pi r^2 L/t$.

Naturally when t_1 is used the rate is somewhat higher than when t_2 is the measure of time. The values obtained are in regeneration units which are termed R. U. in this paper.

It is convenient to assign symbols to the ends and this has been done in Fig. 1 which shows the method of naming the hydranths. D_1, D_2, D_3, etc. are used to designate the distal or oral hydranth of each piece of the stem and are numbered consecutively from distal to

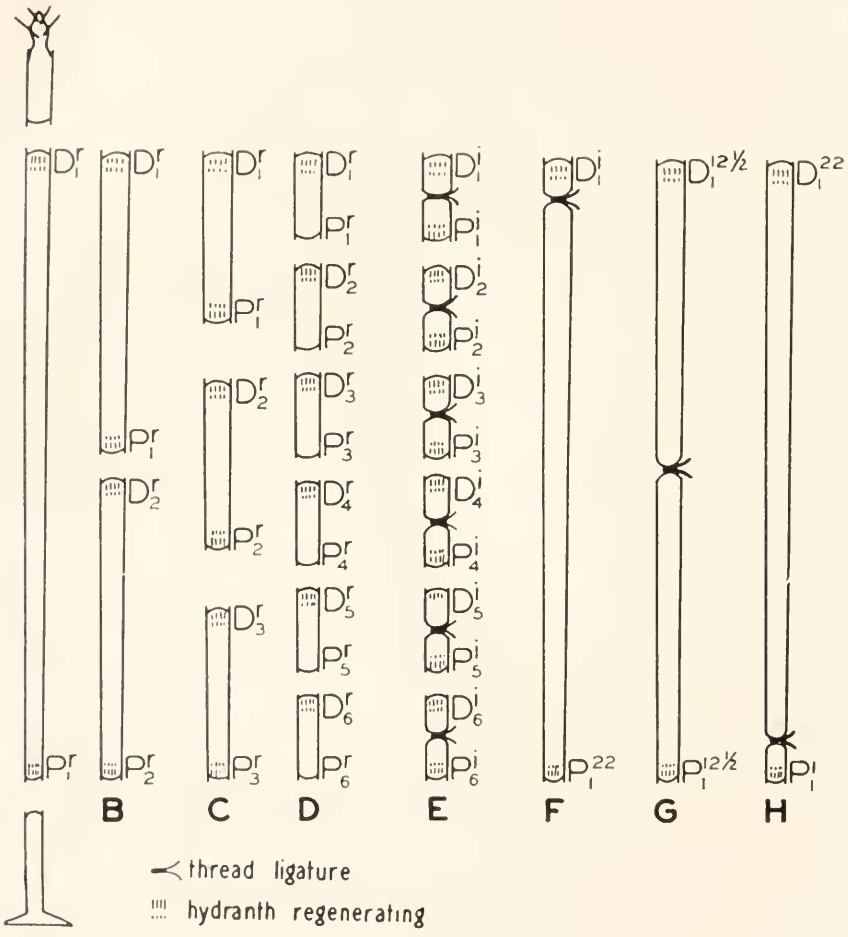

FIG. 1. *A* shows how the stems are prepared; *B*, *C*, *D* how halves, thirds and sixths are prepared and designated; *E* shows how inherent rates are determined by tying off a small part of the stem; finally, *F*, *G*, *H* show how the length of stem is varied while the level remains constant. D_1^r, $D_2^r \cdots$ relative rate of distal hydranths; P_1^r, $P_2^r \cdots$ relative rate of proximal hydranths; D_1^i, $D_2^i \cdots$ inherent rate of distal hydranths; P_1^i, $P_2^i \cdots$ inherent rate of proximal hydranths. D^{22} and P^{22} are examples of absolute rates.

proximal levels. D_1 is always the most distal hydranth of the stem. P_1, P_2, P_3, etc. are used for the proximal or aboral hydranths in the same order. A glance at Fig. 1, *A*, *B*, *C*, will make this clear.

The stems are kept in a rectangular dish which is cooled by a glass coil through which running sea water is circulated. The stems rest on a strip of cheesecloth which is stretched over a glass frame and are covered by one centimeter of sea water. Loose-fitting glass covers are placed over the top of the dish. A glass rod, which is drawn out and bent in the form of a hook, is used for picking up and transferring long stems. For short stems a pipette bent at a 45° angle is satisfactory.

Since the stems of *Tubularia* are very variable in the rate at which they regenerate, controls were prepared for every experiment, and experiments involving the use of different stems are never compared. That is to say, one may not compare the rates of 15-mm. stems from different experiments for they may be vastly different, as measurements show (Table II, Experiments 5, 6 and 7).

The general procedure was to select from several colonies those colonies with straight stems and no side branches. The stems were cut off at the base and placed in a large dish of sea water and further selected for similarity in length, diameter and appearance. Some stems were translucent, while others were opaque, and these two differed in rate of regeneration. After this selection, the hydranths were cut off, and the stems of the same length were selected at random for experimentals and controls. In long stems, the additional precaution of cutting off 3 mm. of the stem along with the hydranth was observed, as the region adjacent to the hydranth often regenerates at a low rate, due possibly to the use of this region in the formation of the very large gonophores.

The Inherent Rates of Regeneration at Various Regions of the Stem

The object of these experiments is to isolate various levels of the stem so that the inherent rate of regeneration can be determined. Isolation of a region from the rest of the stem can be obtained by means of a thread ligature which is tied about the stem shutting off circulation and cutting through the tissue. This technique was used by Morgan (1902) and lately by Peebles (1931). The perisarc, which is tough, does not crack but the coenosarc is completely severed so that there are no cellular connections across the ligature. If this ligature be applied about 2–3 mm. from the end of the stem a small piece of tissue 2–3 mm. in length is isolated and will form a hydranth. By this means the rate of regeneration of a small piece of tissue at any level

of the stem can be measured without being influenced by the stem as a whole. The situation is analogous to self-differentiation of an explant from an embryo. It might be thought that the same result could be brought about more simply by cutting off a piece 2–3 mm. in length. However, in this case one meets with the difficulty that two centers of regeneration arise at the two cut ends and bipolar forms may arise. It is impossible to get any measure of the rate at which these forms develop.

TABLE I

Inherent rates of regeneration of distal and proximal hydranths at various levels of the stem of *Tubularia*. Stems 30 mm. long, cut into 6 pieces and each piece ligatured in middle. L = length in μ, d = diameter of stem in μ, t_1 = time in hours from cutting to the constriction of the regenerating hydranth.

	L	d	t_1	$\frac{L}{t_1}$	$\frac{\pi r^2 L}{t_1} \cdot 10^5$
D_1	1136	504	31.6	36.0	71.6
D_2	1044	488	34.4	30.3	56.4
D_3	928	456	36.4	26.2	43.3
D_4	880	432	39.6	22.2	33.1
D_5	592	388	39.8	14.8	17.6
D_6	404	364	49.1	8.2	8.7
P_1	924	504	39.1	23.6	47.0
P_2	824	456	39.3	21.0	33.9
P_3	844	432	40.9	20.6	30.3
P_4	704	408	47.5	14.8	19.4
P_5	416	372	44.5	9.2	9.6
P_6	244	340	52.8	4.6	4.2

The method of determining the inherent rates of regeneration at various levels of the stem is shown in Fig. 1, E, where the superscript "i" is used to designate inherent rate. Stems 30 mm. in length are first cut into 6 pieces and then each 5-mm. piece is ligatured in the middle. Thus in each 5-mm. stem the distal half is completely isolated from the proximal half. Since the stem which regenerates is so short, 2.5 mm., the hydranths have difficulty in emerging and so the time recorded is t_1. This is the time from cutting to the formation of the primordium of the hydranth.

In Table I and Fig. 2 the data are recorded and plotted. The rates are calculated in two ways. $R_1 = \frac{L}{t_1}$ and also $R_2 = \pi r^2 L/t_1$ where L = length of primordium in micra, r = radius of stem in micra and t_1 = time in hours. From the table it is seen that there is a gradient in the size of the hydranth and the time for regeneration. The rate

of regeneration R_2 of the distal hydranths $D_1 - D_6$ thus falls off sharply from $D_1{}^i = 71.6$ R. U. at the distal end to $D_6{}^i = 8.7$ R. U. at 5 mm. The lowest rate at 0 mm. is $P_6{}^i = 4.2$ R. U. Thus the most distal level of the stem regenerates at almost 18 times the rate of the most

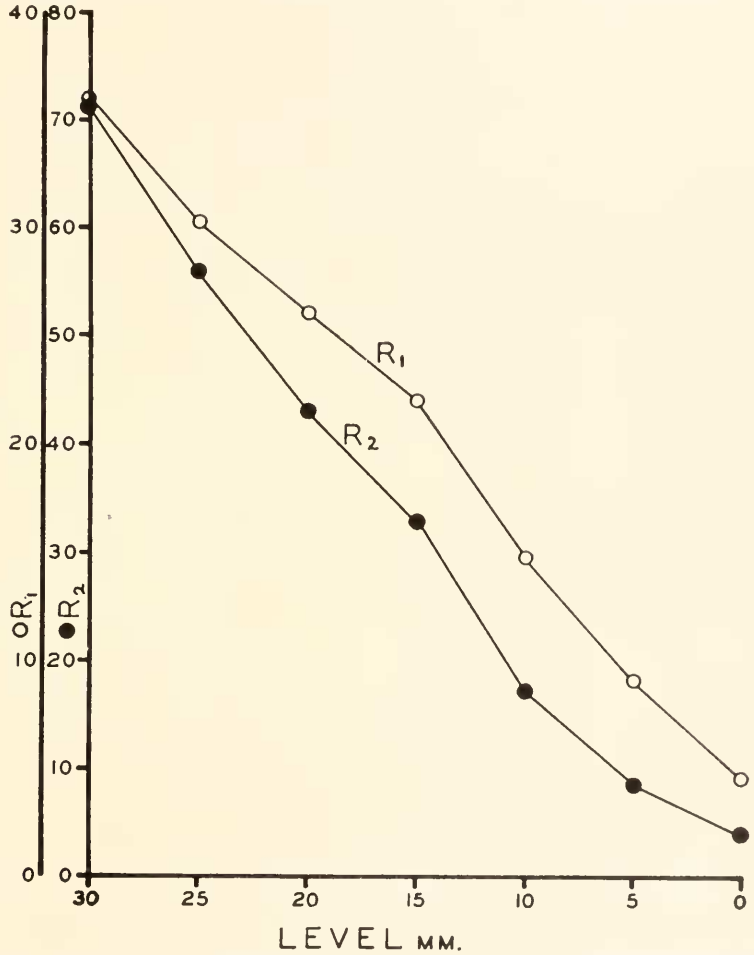

FIG. 2. Rates of regeneration at different levels of the stem of *Tubularia*. $R_1 = \frac{L}{t_1}$; $R_2 = \pi r^2 L/t_1 = \mu^3 \cdot 10^5$/hours. Stems 30 mm. in length cut into 6 pieces and ligatured in middle. Each point is the average of 10 stems. r = radius of cross-section of stem; L = length of primordium; t_1 = time in hours between cutting and the formation of the primordium. The lowest rate is rate of P_6. Zero mm. is at the proximal end of the stem, 30 mm. at the distal end.

proximal level. This difference in rate must be due to causes inherent in the tissue at these levels since external influences have been removed.

The rate of regeneration R_1 has also been calculated using length as a measure of the size of the primordium in place of volume. These calculations are included here to show that the difference in rate of the regenerating hydranths at various levels of the stem is not due merely to a difference in the diameter of the stem but also to a difference in

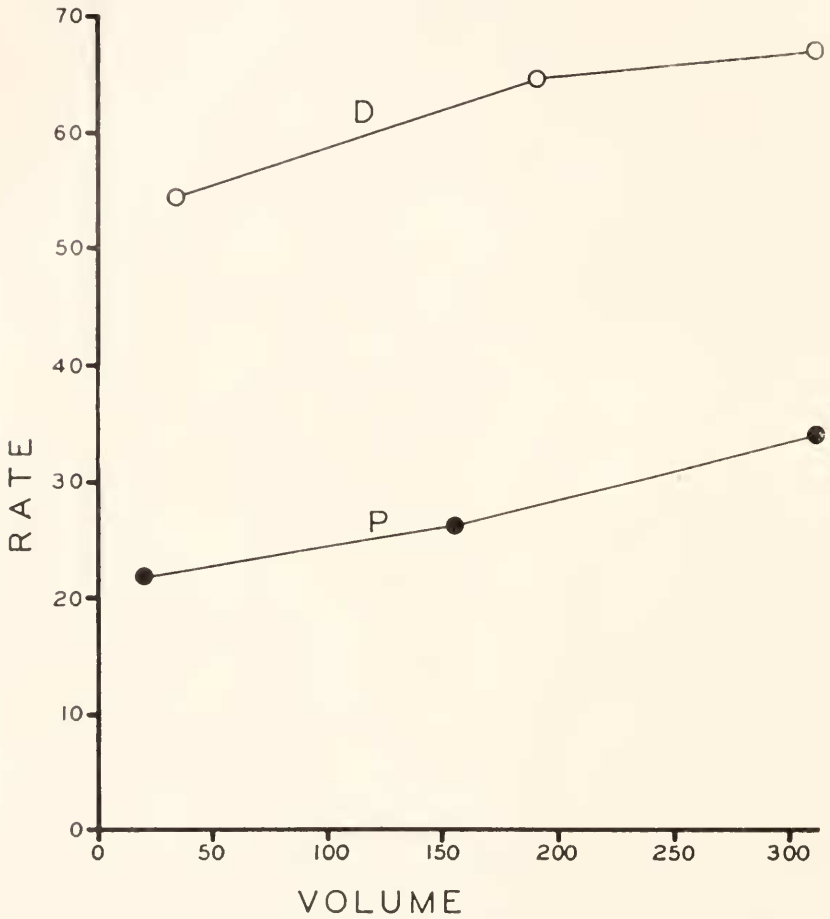

FIG. 3. The effect of volume of stem upon rate of regeneration of hydranth of *Tubularia*. D = distal hydranth; P = proximal. Rate = $\mu^3/\text{hrs.} \cdot 10^5$. Volume = $\mu^3 \cdot 10^7$. Time in this experiment is t_2.

the length of the primordium. What R_1 really represents is the rate of regeneration R_2 divided by the cross-sectional area of the stem and therefore it corrects for the variation in diameter caused by the growth form of the stem. For obviously if the stem is always wider at the distal end as compared with the proximal end, the apparent rate of

regeneration using volume of stem as a measure of size would always be greater at the distal end even if the time for regeneration and the length of the primordium were the same as those of the proximal end. Such a difference in rate seems to me to be entirely due to morphological reasons and would not represent at all the inherent regenerative activity of the tissues themselves.

From the results (Table I, Fig. 2), however, it is clear that the difference in rate at various levels is a real one and that both the time for regeneration and the length of the regenerate vary in different regions of the stem. When R_1 is used as a measure of rate, the difference in distal and proximal levels of the stem is not so great as R_2

TABLE II

Relative rates of regeneration in *Tubularia*. Both hydranths are allowed to regenerate. D^r = relative rate of distal hydranth, P^r = relative rate of proximal hydranth. Rate = μ^3/hours · 10^5.

Experiment	Length	No.	D^r	P^r	Thickness in μ		Vol. Stem
					D	P	
	mm.						$\mu^3 \cdot 10^7$
1	5	6	68.5	0	—	—	—
2	5	21	60.5	0	—	—	—
3	6	25	95.0	4.65	568	528	141
4	10	20	55.0	7.3	488	480	184
5	15	10	40.6	1.9	384	296	136
6	15	10	46.7	18.8	424	368	184
7	15	10	67.0	12.6	476	384	216
8	15	10	70.4	0	560	376	276
9	14	20	93.0	4.9	576	460	296
10	20	10	49.5	19.0	416	344	216
11	25	10	72.5	27.9	460	380	346
12	25	10	67.8	20.4	508	372	380
13	25	10	105.5	29.0	564	424	470

shows. The rate of regeneration of the most distal hydranths $D_1{}^i$ is 36.0 R. U. while that of the most proximal hydranth $P_6{}^i$ is 4.6 R. U., or about an 8-fold difference.

From Table I it will be noted that for pieces of the same length the volume of the stem varies at different levels because the stem is narrower at the base. It might be argued that the reduction in volume of the stem was responsible for the reduction in rate of regeneration. The volume of the most distal 5-mm. piece is $39.4 \cdot 10^7\ \mu^3$, while the most proximal 5-mm. piece has a volume of only $26.0 \cdot 10^7\ \mu^3$. This is a small variation in volume compared to the change in rate from 71.6 R. U. to 4.2 R. U.

The Effect of Length of Stem on Rate of Regeneration of the Ends

Figure 3 shows the effect of varying the volume of the stem but keeping the level of the cut constant; i.e. using the same region of the stem but with more stem attached to it. This is accomplished by a series of ligatures as in Fig. 1, *F, G, H*. Inspection of Fig. 3 tells us that the rate of regeneration at a particular level is increased by increasing the volume of the stem but that the increase is small. For example, increasing the volume from 20.8 to 155 units produces a change in rate from 22 to 26 regenerative units for the proximal hydranths. Further increase of volume to 312 units increases the rate to 34 regenerative units. The distal hydranth behaves similarly.

TABLE III

Comparison of relative and absolute rates of regeneration. D^r = relative rate in $\mu^3/\text{hr} \cdot 10^5$; D^a = absolute rate. Same for proximal. Dominance $= \frac{P^a - P^r}{P^a} \cdot 100$.

Experiment	No. Stems	Length	D^r	P^r	D^a	P^a	Dominance
		mm.					*per cent*
A	6 whole stems	5	45.5	0	—	36.8	100
B	14 distal halves	7.5	40.5	0	38.6	31.9	100
C	14 proximal halves	7.5	23.4	3.9	24.9	15.1	74
D	10 distal thirds	10	52.7	10.7	57.0	35.9	70
E	10 middle thirds	10	36.1	4.4	26.0	13.0	66
F	10 proximal thirds	10	19.1	0	27.0	8.6	100
G	10 distal halves	13	86.7	41.7	90.5	53.0	22
H	10 proximal halves	13	41.4	17.9	53.6	28.5	38
I	10 whole stems	15	67.0	12.6	63.0	26.8	54
J	10 whole stems	15	46.7	18.8	40.4	20.4	7
K	10 whole stems	20	49.5	19.0	51.2	28.9	34
L	10 whole stems	25	105.5	29.0	108.0	47.4	39
M	10 whole stems	25	72.5	27.9	66.8	34.0	20

Compare these small changes in rate with the increase obtained with change in level (Fig. 2). It is of interest here that the total increase in rate for the distal and proximal hydranth, when the 312 units of stem are used, is the same and amounts to about 12 units. This value may express the amount which the middle of the stem contributes to the ends in regeneration.

The Relative Rates of Regeneration of Pieces of Tubularia

Knowing the differences in inherent rates of regeneration and also the effect of volume on rate, we are in a position to study relative rates and the factors that determine these rates. The rate is termed "relative rate" whenever both ends of a piece of stem of *Tubularia*

are allowed to regenerate without a ligature. (See Fig. 1; *A*, *B*, *C*, *D*.) The superscript "*r*" is used. In this case two hydranths are regenerating and are doing so in relation to one another, since they are connected by the stem. Here competition and resultant dominance come in, especially with short stems.

Relative Rates of Whole Stems.—In Table II the relative rates of whole stems of different lengths have been recorded. The stems are prepared as in Fig. 1, *A*. The dominance of the distal regenerating end becomes greater as the stem is cut shorter, so that at 5–10 mm. proximal hydranths often do not appear. As the length of the stem is increased, the rate of regeneration of the proximal hydranth increases. Other factors, such as thickness and volume of stem, play a part.

Relative Rates of Regeneration in Distal and Proximal Halves.—When the stem of *Tubularia* is cut into halves, four hydranths may develop, which we designate as D_1, D_2, P_1 and P_2, as in Fig. 1, *B*. The rates for such stems of various lengths are given in Table III. To show the steepness of the gradient and also the phenomenon of dominance, the relative rates are plotted in Fig. 4. In long stems (26 mm.), there is very little difference in the relative rate of regeneration of the distal hydranth of the proximal half, D_2, and the proximal hydranth of the distal half, P_1. This is to be expected if there is no dominance, since these are adjacent levels of the stem (Fig. 1, *B*). The rapidly developing distal hydranth, D_1, in the distal half does not inhibit the proximal hydranth, P_1, any more than the slowly developing proximal hydranth, P_2, in the proximal half inhibits the distal hydranth, D_2. There is thus no apparent dominance exerted in the ordinary sense, i.e. as an inhibition exercised by a more rapidly regenerating region.

However, in shorter stems dominance appears, and the relative rates of regeneration of the distal hydranth of the proximal half, D_2, and the proximal hydranth of the distal half, P_1, are then quite different. In Fig. 4 the rate of $D_2 = 23.4$ R. U., $P_1 = 0$. In the distal half the distal hydranth D_1 is completely dominant over the proximal P_1. In the proximal half of the stem the distal hydranth, D_2, is only partially dominant over the proximal hydranth, P_2. The rate of $P_2 = 4.0$ R. U.

Finally, in Fig. 4, we have plotted the rates of regeneration of the six possible hydranths, D_1, D_2, D_3 and P_1, P_2, P_3 of Fig. 1, *C* for 10-mm. lengths of stems originally 30 mm. long. The graded differences in rate of regeneration in different regions of the stem are shown by the rates of the distal hydranths and also by the proximal hydranths in Table III, Experiments *D*, *E*, *F*. It will be noted, however, that at any level at which stem is cut the distal hydranth of that level regenerates much more rapidly than a proximal hydranth at the same

level. This means that the distal regenerating hydranths in each third of the stem are inhibiting the proximals in that same third. The situation can be summarized by saying that $D_1^r > D_2^r > D_3^r$ and $P_1^r > P_2^r > P_3^r$, but that $D_2^r > P_1^r$ and $D_3^r > P_2^r$, showing that both D_1 and D_2 are dominant. P_3^r is 0, showing the dominance of D_3.

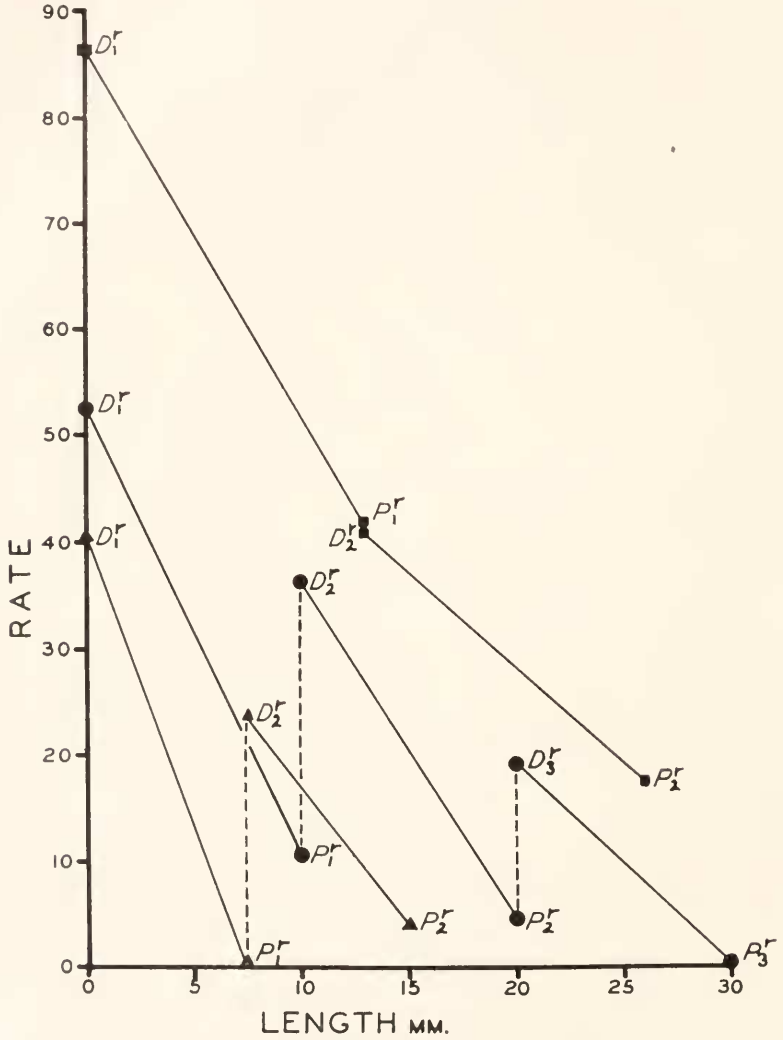

FIG. 4. Relative rates of regeneration of cut stems. Squares = stems 26 mm. long cut into halves; triangles = stems 15 mm. long cut into halves; circles = stems 30 mm. long cut into thirds. D_1^r, D_2^r, P_1^r, etc. = relative rates of respective hydranths. Rate = μ^3/hrs·10^5. The dotted line serves to connect adjacent regions of a cut stem.

The plot of rates in three sets of stems in Fig. 4 shows quite clearly that in each case the gradient is steeper in the upper (distal) levels of the stem. The slopes of the gradient in the upper levels of the three sets of stems are approximately parallel, which seems to indicate that the drop in rate per unit length of stem is about the same in different stems. It should be pointed out that the gradient here will be steeper than the gradient in inherent rates (Fig. 1), since dominance lowers the rate of proximal hydranths.

The Reciprocal Influence of Two Regenerating Regions

In stems of *Tubularia* from 10 to 30 mm. in length, a hydranth usually regenerates at both ends, and it is the purpose of the following experiments to show how the rate of regeneration changes when either the distal or proximal hydranth is prevented from regenerating by means of a ligature allowing the hydranth at the opposite end to regenerate free from dominance. These rates have been termed absolute rates, D^a, to distinguish them from relative rates, D^r. When only one end of a stem is allowed to regenerate, the rate is termed "absolute rate" in contrast to relative rate. This absolute rate of one end can be determined by simply tying off the opposite end of a stem, which prevents regeneration at this end. This rate tends to be the maximal rate of regeneration of a given region since the region has the entire length of the stem affecting its rate and is also independent of a second regenerating region (Fig. 1, *II*, D^{22}). It will also be seen that the absolute rate is simply the inherent rate plus the increase due to the addition of the stem.

The Influence of the Distal Regenerating Region on the Proximal End.—In general (Table III), the shorter the stem the greater the inhibiting effect of the distal regenerating region, and the following cases are arranged according to the length of the stem. In each experiment three lots of stems were used. The first lot regenerated without a ligature, giving relative rates D^r and P^r. The second lot regenerated with the distal end tied off, giving the absolute rate of the proximal end P^a, while in the third group the proximal end was ligatured, giving the absolute rate of the distal end, D^a.

Without exception (Table III), the absolute rate of any proximal hydranth is higher than its relative rate. Indeed, in some cases the relative rate may be 0, while the absolute rate is fairly high, 36.8 units (Experiment *A*). This method of treating the proximal end furnishes a measure of dominance, for if we know the absolute rate and the relative rate we can calculate the percentage reduction due to the distal end, i.e. the inhibition exercised by a dominant region. In

Table III, last column, this percentage has been calculated, and dominance varies from 100 per cent in short pieces to 7 per cent in longer pieces.

The Influence of the Proximal Regenerating Region on the Distal End. —In contrast to the proximal hydranth, the distal hydranth is affected little if at all by the ligature at the proximal end. This is true in the upper levels of the stem. Thus, in Table III, Experiment *B*, the relative rate of the distal hydranth, $D_1^r = 40.5$ R. U., while the absolute rate of the same hydranth, $D_1^a = 38.6$ R. U. and also, Experiment *L*, $D_1^r = 105.5$, $D_1^a = 108.0$. However, in lower levels of the stem, the absolute rate of the distal hydranth is usually greater than the relative rate. Experiment *F*, the relative rate of the distal hydranth of the

TABLE IV

The relation of the inherent rates of regeneration to the relative rates in pieces of *Tubularia* of varying length. Rate $= \frac{\mu^3}{t} \cdot 10^5$. D^i = inherent rate of distal hydranth; P^i = inherent rate of proximal hydranth; D^r = relative rate of distal hydranth; P^r = relative rate of proximal hydranth.

Length	Ligature D^i	Ligature P^i	No Ligature D^r	No Ligature P^r	Ratios D^i/P^i	Ratios D^r/P^r	Remarks
mm.							
25	54.6	22.0	72.5	27.9	2.46	2.60	Chiefly partition, no dominance.
20	38.2	13.9	49.0	19.0	2.74	2.60	Chiefly partition, no dominance.
14	81.5	22.6	93.0	4.9	3.61	19.0	Dominance incomplete.
5*	71.6	47.0	78.0	0	1.53	∞	Dominance complete.

* t_1 used in place of t_2.

proximal third of the stem $D_3^r = 19.1$, the absolute rate of the same hydranth, $D_3^a = 27.0$; Experiment *H*, $D_2^r = 41.4$, $D_2^a = 53.6$. This is extremely interesting, because it shows that the proximal hydranth does have an inhibiting influence over the distal hydranth in lower levels of the stem. In proximal regions of the stem then there is a reciprocal influence of the two regenerating ends on each other, each tending to inhibit the other.

The Competition between Two Regenerating Regions Having Different Rates

In the second section of this paper it has been shown that adding parts of the stem to either the proximal or distal end increases the rate of regeneration. Therefore the stem as a whole contributes materials to the regenerating ends, and the way in which this material is par-

titioned can be studied in stems of different lengths which provide different amounts of the material. The following experiments were designed to determine how the distal and proximal hydranths would divide or partition the effect of adding a definite volume of the stem to the system. For this purpose the inherent rates of regeneration of the proximal and distal hydranth were found by tying 3-mm. ligatures at the ends of the stems as in Fig. 1, *F* and *H*, D_1^i and P_1^i. In the same stems the opposite ends D_1^{22} and P_1^{22} give the rates of regeneration with 19 mm. of stem added to each end. Finally, as in Fig. 1, *A*, the two ends were allowed to regenerate in competition for the intervening 19 mm. of stem.

It is seen from Table IV that in long stems (20–25 mm. lengths) the addition of the middle of the stem to the distal and proximal ends

TABLE V

Increase in rate of regeneration of distal and proximal ends when the same volume of stem is added to each.

Total Length	Stem 3 mm. Inherent Rate		Volume Added $\mu^3 . 10^7$	Stem 22 mm. Absolute Rate	Increase in Rate
mm.					
25	Distal	54.6	280	66.8	12.2
	Proximal	22.0	266	34.0	12.0
	Stem 3 mm. Inherent Rate			Stem 17 mm. Absolute Rate	
20	Distal	38.2	163	51.2	13.0
	Proximal	13.9	176	28.9	15.0

increases the rate of regeneration of these ends in direct proportion to their inherent rates. That is, the relative rates are directly proportional to the inherent rates $D^r/P^r = D^i/P^i$. Therefore there is simply a partition of materials without any dominance. This result would be expected if no factor other than available foodstuffs controlled the rates of regeneration.

However, in short stems (14 and 5 mm. lengths) there is no longer a partitioning of substances in proportion to the inherent rates of the distal and proximal hydranths, but rather a dominant effect of the distal end, so that it takes more than its share. $D^r/P^r > D^i/P^i$. As a matter of fact, in all non-ligatured 5-mm. pieces examined in this experiment and many others, no regeneration occurred at the proximal end at all, in spite of the fact that the proximal end had an inherent rate almost as great as the distal $D^i/P^i = 1.53$. This situation represents complete dominance in which all of the material goes to the distal hydranth.

A Comparison of the Effect of the Distal Half and the Proximal Half on the Inherent Rate of Regeneration of the Ends

It has been shown that the middle regions of the stem contribute materials for the regeneration of the two ends. The amount which the stem contributes can be measured by the increase in the rate of regeneration of the hydranths under the assumption that rate is proportional to amount of substances available. Thus, in Table V, the inherent rate of regeneration of the distal end is 54.6 R. U., and when we add 19 mm. of stem, the rate increases to 66.8 R. U. or an increment of 12.2 R. U. Similarly, P^i increases from 22.0 to 34.0 R. U., or an increase of 12.0 R. U. The increase is the same for both proximal and distal ends. (See 20-mm. stems also.) It is clear that the materials of the stem can be used by either the proximal or distal end.

Now if we ligature the stem in the middle, the effect of the materials in distal and proximal halves on the regeneration of the ends may be

TABLE VI

Comparison of the effect of distal and proximal halves of a stem on the rate of regeneration of the ends. D^i is the inherent rate of regeneration of the distal end. Under distal half is the rate of the distal end, with middle ligature. Similarly P^i is the inherent rate of the proximal end. Proximal half = rate of proximal end with middle ligature.

	D^i	Distal Half	Increase	P^i	Proximal Half	Increase
25 mm.	54.6	64.6	10	22	26	4
14 mm.	82.0	98.0	16	22.6	24.2	1.6

studied (Table VI). It is found in the two experiments available that the increase in rate is much greater in the distal as compared with the proximal half. This may be taken to mean that there are more materials available in the distal half than the proximal. The curves for the effect of volume on rate (Fig. 3) also indicate that the distal half is more effective in increasing rate than the proximal. As volume is added to the distal end, the rate goes up sharply and then falls off. However, as volume is added to the proximal end, the rate goes up slowly at first and then sharply. The evidence seems rather conclusive that the two regions differ in their effects on rate of regeneration.

Isolation of Distal and Proximal Regions by Means of a Middle Ligature and its Effect on the Relative Rate of Regeneration of the Ends

This method of studying dominance was used in the first experiments, and it is complicated by the fact that not only is isolation pro-

duced by the ligature but the volume of the stem is also reduced. The effect on the proximal hydranth is a dual one. Isolation increases the rate of regeneration, while reduction in volume decreases the rate, and the effect is a summation of the positive and negative action. Since, as we have shown, the reduction of the volume of the stem adjacent to the proximal hydranth decreases the rate to a small extent only, the chief effect is to remove the dominant region from the sphere of action, and the net result is an increase in the rate of the proximal hydranth (Table VII).

Length is an important factor, as in 25-mm. stems the change in rate with ligature is slight, while in 15-mm. stems it may be much greater, depending on the condition of the stems. This is in keeping with the fact that little dominance is exerted in long stems.

TABLE VII

Rate of regeneration of distal and proximal hydranths of *Tubularia* with and without a middle ligature. D^r and P^r = relative rate; D^l and P^l = rate under conditions of middle ligature.

Experiment	Length	No.	D^r	D^l	P^r	P^l
	mm.					
1	25	10	67.8	70.5	20.4	25.4
2	25	10	72.5	64.6	27.9	26.0
3	15	10	46.7	43.2	18.8	21.0
4	15	10	63.0	64.0	17.5	23.0
5	15	10	40.6	36.4	1.9	15.0
6	15	10	47.6	38.6	0	15.1
7	14	20	93.0	98.0	4.9	24.2

The Mechanism of the Dominance Exerted by the Distal Regenerating Hydranth over the Proximal End of the Cut Stem

In all previous experiments a ligature was used to block the dominance exerted by the distal regenerating end over the proximal end. With ligature of the stem the proximal end is allowed to regenerate independently of the distal and its rate of regeneration is greatly increased. This ligature, however, not only stops circulation between the distal and proximal end but also severs cellular connections. Thus it is not clear whether the factor responsible for dominance is something present in the circulation or something transmitted or transported through the cells. Therefore it is necessary to determine the effect on the proximal end of stopping circulation from the distal end but leaving cellular connections intact.

Use of a Loose Ligature for Blocking Circulation.—It is not easy to

shut off circulation between parts of the stem of *Tubularia* as the cells will rearrange themselves after compression of the stem so that the circulation breaks through once more. However, there are indications which can be seen from Table VIII where the effects of a loose ligature which was tied so as to just stop circulation is compared with a tight ligature cutting through the cœnosarc and breaking all cellular connections. The control with no ligature shows that we are dealing with stems in which the distal end is almost completely dominant over the proximal end: i.e. distal end, 38.7 R. U.; proximal end, 2.9 R. U. A tight ligature completely isolating the two halves sends the rate of regeneration of the proximal end up to 23.0 R. U. or an 8-fold increase. The distal end shows a slight reduction to 34.0 R. U. as it is cut off from the proximal half of the stem. Now with a loose ligature where cellular connections are still intact the proximal end shows an increased rate of 23.0 R. U. over controls in spite of the fact that at the end of the experiment circulation was reëstablished in a few cases through

TABLE VIII

Rate of regeneration of distal and proximal ends of *Tubularia* under conditions of ligature in the middle of the stem. Rate = L/t_2 where L is length of primordium in micra and t_2 is time in hours from cutting of stem to emergence of hydranth.

No. and Length of Stems		No Ligature		Tight Ligature		Loose Ligature	
		D	*P*	*D*	*P*	*D*	*P*
10	15 mm.	38.7	2.9	34.0	23.0	36.4	23.0

the ligature. This sort of experiment, while it appears conclusive, is not entirely satisfactory as it is difficult to control the tying of a ligature so as to cut off circulation without breaking cellular connection between the two halves.

Injection of Oil to Block Circulation.—The stem of *Tubularia* is about 0.5 mm. in diameter and it is relatively easy to insert a micropipette for injection. It is necessary merely to crack the rigid perisarc first with two pairs of sharp watchmaker's forceps after which a pipette can be inserted while observing under a binocular microscope. A small drop of paraffin oil (Nujol) is injected and after the pipette is withdrawn the rigid perisarc snaps back into place. The perisarc must not be removed because then regeneration will take place at the exposed surface. Controls for this type of experiment were stems in which the perisarc was ruptured and the pipette inserted without injection. Some of the experiments where oil was injected also served as controls since the drop was sometimes too small to block circulation.

Table IX records results. There are 25 stems in each sample and of the 25 controls only 2 proximal hydranths developed, making the rate 4.65 R. U. and thus showing that the distal end exerted rather complete dominance. When circulation is blocked, however, the 25 injected stems regenerate 12 proximal hydranths, bringing the average rate to 22.8 R. U., or a 5-fold increase in rate. The distal hydranth in the injected group shows a small decrease in rate, as might be expected from previous results on the use of ligatures. A ligature in a short stem increases the rate of the inhibited proximal end but decreases the rate of the dominant distal end by shortening the stem.

Only 12 out of 25 possible hydranths appear at the proximal end of injected stems and it is interesting to examine the 13 stems which did not form a hydranth proximally. Of these 13 negative cases 9 showed that the oil drop had moved from its original position at the middle of

Table IX

Injection of an oil drop into the gastrovascular cavity of *Tubularia*. Stems 6 mm. long. Twenty-five stems used in each sample. R = rate of regeneration $= \pi r^2 L/t_2$ where r = radius of stem in micra; L = length of primordium in micra and t_2 = time in hours required for emergence of the newly regenerated hydranth.

	Oil Injected		No Injection	
	Distal	Proximal	Distal	Proximal
L	1384	528	1536	104
r	282	264	284	280
t_2	40.0	50.5	40.9	55.0
R	86.1	22.8	95.0	4.65

the stem into the distal end. In the 12 stems which form a proximal hydranth all but 2 showed the oil in the original position. It is clear that because the size of the oil drop varies the smaller drops do not completely block circulation and as a result they are carried by the circulation to the distal end. When the drop is larger it is held firmly in place by the endodermal lining of the gastrovascular cavity and in these cases proximal hydranths appear associated with a complete block to circulation.

In other experiments where dominance is not so complete there is a quantitative effect on rate of regeneration of the proximal end, with injection of oil into the gastrovascular cavity. It must be remembered that this effect is not so great as would be expected since not all of the oil drops are large enough to block circulation. An example is shown in Table X, where the proximal ends of control stems regenerate at a

TABLE X

The effect of blocking circulation in *Tubularia* with oil drops. Oil injected into distal region. Controls consist in injury similar to that of injection. Twenty-one stems, 10–11 mm. in length used for each sample. $R = \pi r^2 L/t$.

	Oil Injected		Injury Control	
	Distal	Proximal	Distal	Proximal
L	1200	1032	1352	612
r	254	236	252	228
t_1	35.4	47.0	37.3	50.0
R	67.0	38.4	72.0	20.0

rate of 20.0 R. U., but upon injection of oil increase to 38.4 R. U. The rate of the distal hydranth is reduced from 72.0 R. U. in untreated

TABLE XI

Effect of blocking circulation by means of oil in *Tubularia*. Stems 8–10 mm. long isolated from long thick stems. Twenty-eight stems in each sample. R = rate using t_1; l = length of primordium in micra; r = radius of stem in micra; t_1 = time in hours from cutting to formation of primordium. Rate = μ^3/hrs.$\cdot 10^5$.

	Oil		Control	
	Distal	Proximal	Distal	Proximal
L	1388	884	1320	852
r	286	276	286	268
t_1 hours	37.8	46.9	37.3	46.0
R	94.0	45.0	91.0	41.7

stems to 67.0 in injected stems. This decrease is in part caused by injection of the drop into the distal region of the stem which isolates a small portion. The average length of stem from the oil to the distal end was 2.5 mm. at the termination of the experiment.

Finally, in stems where there is very little dominance there is little effect of injection of oil in the middle of the stem. This result was obtained from some 8–10 mm. pieces cut from long, thick stems. Table XI shows that neither the proximal nor distal hydranths are affected to any extent by the injection of oil. This result is to be expected from the section dealing with the use of a middle ligature to isolate the two ends. In stems where the distal hydranth exerts little dominance it was found that little effect was produced on the rate of regeneration of the proximal hydranth by a ligature.

The experiments on injection of oil into the gastrovascular cavity

of *Tubularia* shows that the oil isolates the proximal end of the stem from the distal end, producing about the same effect as a ligature. In the former case the circulation is blocked while in the latter both circulation and cellular transmission are blocked. It becomes important then to see just what the oil drop does in the gastrovascular cavity. It has already been pointed out that when the drop is small it has little effect in blocking dominance. Also, when there is little dominance, little or no effect of injection of oil is found. Therefore it is safe to say that there are no toxic chemical or physical effects of the oil on the cells which come in contact with the oil.

Examination of the region into which the oil is injected shows it to be firmly held in place by the endoderm which it partially displaces. When it is not so held in place it moves during the course of hours to the distal end of the stem. Although the endoderm is displaced and perhaps the cellular connections in this layer are broken, the ectoderm remains intact and the cells can be seen to be continuous over the surface of the oil drop. As we were not satisfied with this observation, the conductivity over the bridge of ectoderm was tested by using an electrical stimulus. In some previous unpublished work on electrical stimulation in *Tubularia* it was found that upon applying a stimulus at the proximal end the tentacles of the distal hydranth would respond. Three stems in which dominance was blocked by injection of oil were treated in this manner and in each case the tentacles of the distal hydranth responded to an electrical stimulus applied at the proximal end. From these observations there can be little doubt that the ectodermal connections over the oil drop are morphologically and physiologically intact and that dominance is not transmitted over this layer.

Discussion

Child (1907) pointed out that in *Tubularia mesembryanthemum* both the length of the cut stem and the level at which the stem was cut were factors determining the time for regeneration and size of the primordium. Driesch (1899) before this had measured the primordium of halves of stems and found that the oral half (distal half) formed longer primordia than the aboral (proximal half). Driesch also showed that the hydranths emerged faster in the oral (distal) half as compared with the aboral (proximal) half. Thus the regional differences in regenerative capacity in the stem of *Tubularia* are by no means new. The new treatment of the facts by combining two variables, size and time, into a rate has not been suggested hitherto. By utilizing both variables it is possible to express the rate of change within the stem at any level and so compare rates under various conditions. It is hoped that the

rate as defined in this paper is a measure of the chemical changes involved in the differentiation of the hydranth from the stem after cutting. It is not sufficient to express these changes in terms of time only since two hydranths of different sizes may regenerate in the same time and certainly the larger hydranth must have utilized more material than the smaller. Therefore the rate of chemical change must have been higher in the region which formed the larger hydranth. Similarly two hydranths of the same size may require different times for regeneration.

A second difference between these experiments and those of early investigators is the use of a ligature to isolate regions in order to test their rate of regeneration. Driesch (1899) and Child (1907) cut stems into halves and thirds and allowed both ends to regenerate. The size and time for regeneration of distal (oral) hydranths is modified by the presence of a second region of regeneration. Both investigators showed that the size and time factors varied with the length of stem cut. In my experiments by the use of the ligature the second regenerating region is eliminated and the size kept constant. Thus the "inherent" rate is measured. It is proposed that the "inherent" rate of regeneration be used as a base so that the effect of variables such as length of cut stem and the rate of regeneration of a second hydranth can be studied by means of appropriate ligatures.

Since the stem of *Tubularia* shows a gradient of "inherent" rates there must be graded differences in the concentration of some substance or substances, which account for these different rates. Further, because the rates are higher in the younger (distal) regions of the stem it is reasonable to assume that the substances are of the nature of a synthetic factor which is able to convert available materials into a hydranth.

We will let this inherent synthetic factor be represented by E and assume that the concentration of E is proportional to the inherent rate of regeneration as measured by isolation of parts of the stem. E is then present in highest concentration in the young cells at the distal end and in lowest concentration at the proximal end. Then Fig. 2, giving rates of regeneration, may be taken to indicate the relative concentration of E at various regions of the stem since only internal factors are responsible for these differing rates at various levels of the stem.

But E is not the only factor affecting rate. In Fig. 3 it was shown that increase in length of stem adjacent to the regenerating region will also increase rate of regeneration. It is evident that something from the middle of the stem travels to the ends and causes an increase in rate. Let us call this factor or substance S. S is transported through

the gastrovascular cavity in the circulation which is easily observed in *Tubularia*. A cross-section of the stem shows four channels in the endoderm and in the intact stem particles can be seen travelling up one side of the stem and back down the other so that a fairly rapid circulation exists. Timing the flowing particles gave a velocity of 6 mm./minute. This means that in a short stem 6 mm. long a complete circulation of the contents of the coelenteron should take place in 2 minutes. If one end was using up materials in rapid regeneration it is conceivable that the concentration of substance, S, in the circulation would be lowered considerably so that at the opposite end substances might pass into the gastrovascular cavity from the cells and so inhibit regeneration by removal of available materials.

The effect of S on rate of regeneration is difficult to measure but we may take Fig. 3, which shows increase of rate with increased volume of stem as a provisional measure of S. Obviously, however, this does not give us the effect of S in very low concentrations. That S is a very important factor is seen by comparing the inherent rate of the proximal end with the relative rate (Table IV, 14-mm. and 5-mm. stems). In these cases something is actually removed from the proximal region so that although the stem is much larger the rate of regeneration is lower when the distal hydranth is regenerating.

The situation may be summarized as follows. Regeneration is essentially the transformation of stem into hydranth and this requires chemical changes: $S \overset{E}{\underset{\downarrow}{\rightleftarrows}} II$. Let us assume E to be a catalyst in the cell which transforms S into II, II being the substances necessary for hydranth differentiation. The reaction is reversible, as Child (1923) has shown that hydranths may dedifferentiate into stem. I have also observed a hydranth partially differentiate from cœnosarc and then return to cœnosarc. E, as we have pointed out, is present in the cells and is in highest concentration at the distal end. S is in the cells and also the gastrovascular cavity and is present in greatest amount in the longest stems.

In long stems, where S is high (Table IV, 25-mm. and 20-mm. stems) it appears that S is partitioned to the distal and proximal hydranths according to their inherent rates of regeneration, as represented by E. One way of expressing it is that, with increase in S while Ep (concentration of E at the proximal end) and Ed (concentration of E at the distal end) remain constant, II_p (concentration of II at proximal end) and II_d (concentration of II at distal end) increase proportionally. This result is the expected one.

The difficulty comes when we consider short stems, where E_p (concentration of E at the proximal end as measured by the proximal inherent rate) is close to E_d (concentration of E at the distal end as measured by the distal inherent rate) yet apparently S is used by the distal hydranth and not by the proximal hydranth. All that can be assumed is that the factor E_d converts S into H as fast as it appears in the cœlenteron, so that the concentration of S is always below the minimal value necessary for regeneration at the opposite end. Since $E_d > E_p$ it can synthesize H at a lower concentration of S. By writing the reaction as a reversible one, we may even suggest that $H \rightarrow S$ at the proximal end in the case of short stems. This whole thought depends on the assumption that the difference between E_d and E_p in short stems, although small, is great enough to lower the concentration of S below a minimal value, S_m for the proximal hydranth. As S increases in amount (in longer stems) the concentration in the cœlenteron rises above the minimal value S_m for the proximal hydranth and regeneration starts at the proximal end though at a low rate at first. S thus becomes a limiting factor for regeneration in low concentrations.

It will be seen that the formal explanation given above is readily applied to other results, such as those from middle ligatures and the results showing the difference between relative and absolute rates of regeneration.

From the comparison of the absolute rate of the distal hydranth D^a and the relative rate of the same hydranths D^r in Table III, it is apparent that there is a maximal rate of regeneration for the distal hydranth, or a maximal concentration of S above which synthesis of H is not increased perceptibly. This is not unlikely.

The above discussion throws the entire effect of dominance on the transport of available substances (S) for regeneration. In *Tubularia* the transport system is extremely simple, and it seems an ideal system for experimentation. If in short stems the circulation of S is blocked (as by an oil drop) both the distal and proximal ends should regenerate independently of each other and no dominance will be exerted by the distal end. The experiments in this paper indicate that such is the case and thus show that S is a factor which circulates in the fluid of the gastrovascular cavity.

Summary

1. The rate of regeneration of *Tubularia* has been measured by the formula $R = \pi r^2 L/t$ where r = radius of cross-section, L = length of regenerate, t = time in hours for formation of primordium or the time in hours for emergence of the fully formed hydranth.

2. The rate of regeneration of isolated parts of the stem decreases

rapidly from the distal to more proximal regions. An increase in the length of stem adjacent to a regenerating end increases the rate of its regeneration.

3. When two regenerating regions are competing they partition the effect of adding the middle region of the stem if the stem is long. If the stem is short the distal end becomes dominant and inhibits the proximal end.

4. A method has been devised for measuring dominance by expressing it as the percentage reduction in rate of regeneration due to the presence of the distal hydranth.

5. The circulation within the stem of *Tubularia* has been blocked by means of injection of a drop of oil with the result that the dominance (inhibition) exercised by the distal regenerating end over the proximal end is blocked.

6. A generalized mechanism based on a synthetic factor E in the cells and a circulatory factor S is suggested as a formal explanation of the phenomenon of dominance.

LITERATURE CITED

CHILD, C. M., 1907. An analysis of form regulation in Tubularia. IV. Regional and polar differences in the time of hydranth formation as a special case of regulation in a complex system. *Arch. Entw.-mech.*, **24**: 1.

CHILD, C. M., 1923. The axial gradients in Hydrozoa. *Biol. Bull.*, **45**: 181.

CHILD, C. M., 1929. Physiological dominance and physiological isolation in development and reconstitution. *Arch. Entw.-mech.*, **117**: 21.

DRIESCH, HANS, 1899. Studien über das Regulations-vermögen der Organismen. *Arch. Entw.-mech.*, **9**: 103.

MORGAN, T. H., 1902. Further experiments on the regeneration of Tubularia. *Arch. Entw.-mech.*, **13**: 528.

PEEBLES, F., 1931. Some growth-regulating factors in Tubularia. *Physiol. Zoöl.*, **4**: 1.